EXHIBIT A

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PACSIMILE TRANSMITTAL SHEET

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FROM: MARK
COMPANY: Palo Alto
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NOTES/COMMENTS:

Comment, thoughts, corrections, improvements please

Let's discuss next week

Mark

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Invention Disclosure

Based on discussion between Tom Brennan and Mark Berninger (related to ideas proposed by Francois Chatelain) initiated on in Palo Alto.

Title

Use of hydrophobic/hydrophilic surface arrays to deliver oligonucleotides to multiple reaction volumes, particularly nucleic acid amplification reactions.

Background

Within the field of molecular genetic research there exists a need to carry out large numbers identical or similar separate amplification reactions in parallel. Currently the cost in terms of time, labor, equipment, laboratory space and reagents is prohibitively high for many laboratories. Further it is necessary to analyze the products of each separate amplification reaction. This also entails high, often prohibitively, high costs. A prime example of this need involves the genetic study of human populations to understand the genetic basis of human disease and the variable response among humans to medical treatments including drugs.

In populations such as humans there exist many genetic loci, which vary among individuals, termed polymorphic sites, which are useful in the genetic analysis of disease, risk of disease or response (including toxic response) to chemicals, including drugs. Among the most useful of these polymorphic sites are single nucleotide polymorphisms (SNP's) in which individuals differed by a single nucleotide at a particular site. Genetic analysis using SNP's commonly requires many (up to 30,000) SNP's to be analyzed per individual. As SNP's are dispersed around the genome it is necessary to amplify a comparable number of discrete regions in the genome so that each SNP can be analyzed. Accordingly the genetic analysis of a single individual's SNP's (genotyping) can require that up to 30,000 amplifications be carried out (optimally in parallel) and the product of each amplification be analyzed. Genetic analysis of a disease may require the extensive genotyping of hundreds or thousands of individual humans. The number of separate amplifications and analyses can therefore number in the millions.

A technology has been developed by Protogene Laboratories Inc. by which precisely defined region of hydrophilic surface can be generated in precise patterns on against a hydrophobic surface background using photolithography. This technology has been used to facilitate the in situ synthesis of oligonucleotides within the hydrophobic regions of these arrays using glass as a substrate. These same hydrophilic regions have also been used as sites of deposition for pre-synthesized oligonucleotides that are then covalently bound to the glass within these sites. The fact that the hydrophilic regions on the surface contain the applied aqueous solutions within their boundaries, thereby segregating each such region from neighboring regions by virtue of the hydrophobic regions between neighboring hydrophilic regions, have enabled the precise and reliable location of oligonucleotides on the surface of the array.

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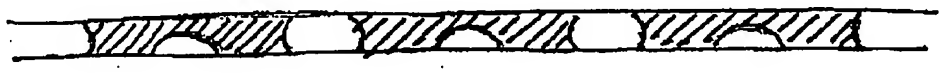
It is also possible to effect a similar bridging of aqueous solution between two or more spots of an array by bringing a surface having an appropriate pattern of hydrophilic and hydrophobic regions into apposition with the array. An example is shown below:



Use of cleavable sites to release immobilized amplification primers

Within in each hydrophilic region or spot of the array a particular oligonucleotide can be synthesized on the surface. Alternatively, one or more pre-synthesized oligonucleotides can be bound to the surface of such a region or spot. All or some of these oligonucleotides also can be synthesized with cleavable sites (e.g. uridine by UDG and *E. coli* Endonuclease IV) thereby allowing their release into solution by the cleaving agent.

Efficient nucleic of nucleic acids using, for instance, PCR requires that a sufficient concentration of primers be free in solution, and that (in the case of PCR) at least two primers be present in the same solution and be in contact with the amplifiable sequence. Two immobilized oligonucleotide primers can, therefore be brought into contact with PCR reaction components and then subsequently released from the surface into solution to mix with one another amplifiable DNA and the other reaction components and initiate amplification. It is known that it is not necessary for amplification primers in PCR to be present at equimolar concentration. When they are not at equimolar concentrations the PCR is said to be asymmetric and an excess of one of the DNA strands is present in the reaction product. It is not necessary to necessarily release all of the immobilized oligonucleotide to support amplification. A fraction of the immobilized oligonucleotide may be left immobilized to capture through hybridization products of amplification, particularly if the PCR is asymmetric.

Application to amplification of genomic DNA for genetic analysis.

Shown below is a configuration of oligonucleotide primers on a small number of spots of an array of hydrophilic spots against a hydrophobic background, how that array is used to assemble separate PCR's.

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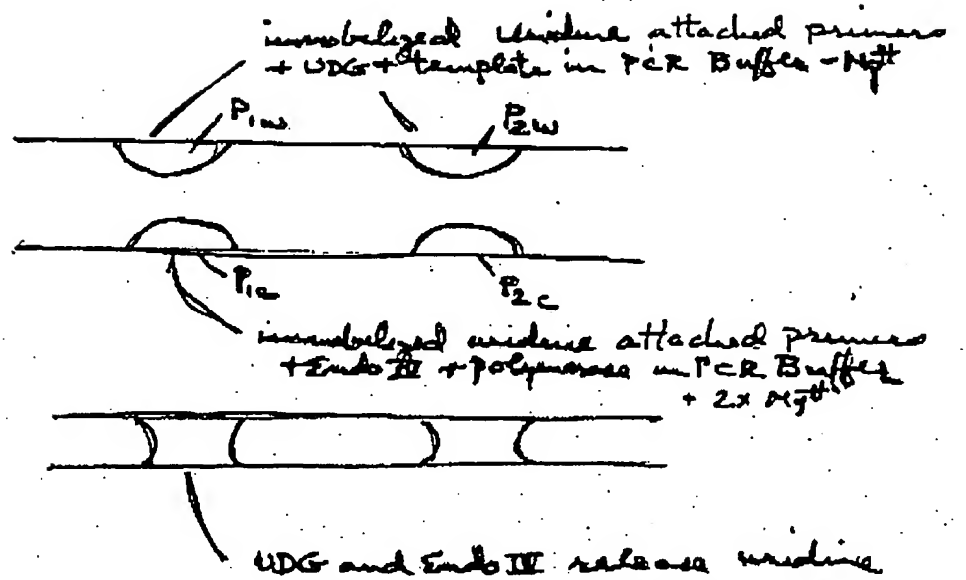
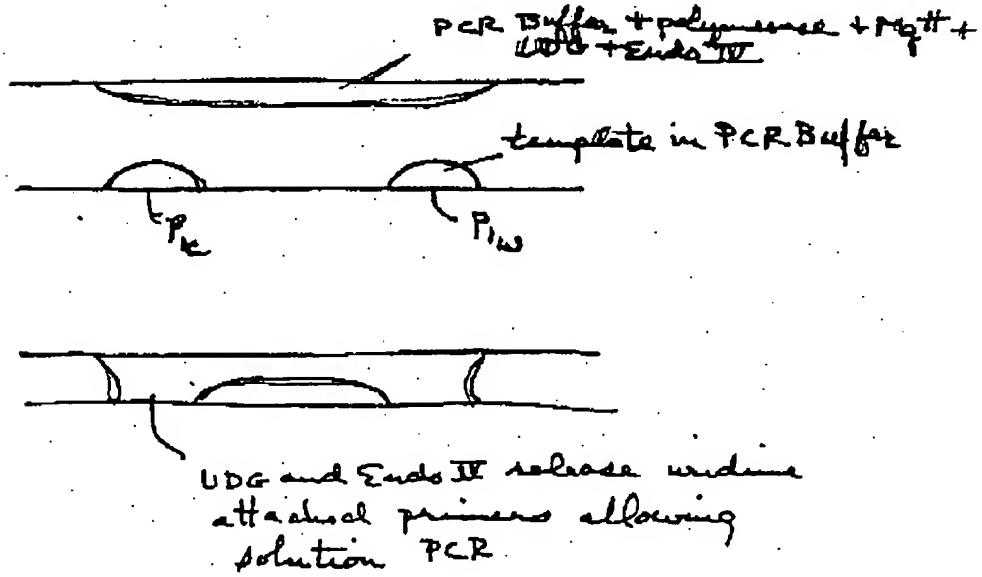
Configuration 1



Configuration 2



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A single solution of genomic DNA, buffer, DNA polymerase, and, for instance UDG and *E. coli* endonuclease IV, in the example in which uridine is used is a cleavable site, can be spread over the arrays to distribute the same amplifiable DNA among many spots each containing a single primer. To delay release and possible mixing among primers this may preferably be done at low temperature to inhibit the enzymatic activity of the UDG and Endonuclease IV. Preferably, two solutions, neither of which alone can carry out PCR or release immobilized primers into solution can prepared and one can be spread over one plate and the other over the other plate as exemplified in the figures above. With the amplifiable DNA so distributed separate reaction volumes may then be created by bring two matched surfaces into close apposition as shown in one of the above diagrammed examples thereby bringing the appropriate two primers into the same reaction volume. Upon action of the UDG and *E. coli* endonuclease IV as in the above example primer are released into solution and PCR may then be initiated by thermocycling the solution between the surfaces using, for example, thermocyclers adapted for *in situ* PCR.

It may also be desirable to have some a fraction (e.g.10% to 20%) of the oligonucleotides in each spot synthesized without uridine so they are not released to participate as primers, but can remain tethered to the surface to capture amplicons. Such amplicons may then be interrogated with respect using other probes to determine polymorphisms for example by single base extension.

An example of how uridine containing primers can be selectively released by the sequential action of UDG and Endo IV is diagrammed below.

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It is also known that DNA oligonucleotides can be synthesized using bases other than adenine, thymine, cytosine, and guanine (e.g. uridine), and can be synthesized with ribonucleotides interspersed among the deoxyribonucleotides. By doing this it is possible to construct oligonucleotides which are covalently bound to a surface such as glass, but which can be released in whole or in part by agents which cleave the oligonucleotide at a base other than adenine, thymine, cytosine, or guanine (e.g. uridine using uracil DNA glycosydase and *E. coli* Endonuclease IV) or at a ribonucleotide.

New methods that will substantially reduce all or some of the above costs (time, labor, equipment, space, reagent, etc.) are badly needed to accelerate research and allow the application of this genetic research to clinical medicine.

Description of the Invention

Hydrophilic/Hydrophobic Arrays to Assemble and/or Segregate Amplification Reactions

When aqueous solutions are applied to hydrophobic/hydrophobic arrays that consist of circular spots of hydrophilic surface against a hydrophobic background beads of aqueous solution form of definable and reproducible volume and position form and which are not affected substantially by gravity when the spots are smaller than about one millimeter in diameter. Such beads are shown below:

If two flat or complementary spaced surfaces with matching hydrophilic and hydrophobic regions are brought into close apposition it is possible for a bead to span the space between the matching regions such that the solution in the bead (that now bridges the space between the surfaces) is simultaneously in contact with hydrophilic regions of both surface. An example of this is shown below.